

## 97580 Mueller Hinton Agar 2 (M-H Agar 2, Mueller Hinton II Agar)

Mueller Hinton Agar 2 is a solid medium recommended by Bauer et al. and NCCLS for susceptibility testing of pathogens. Antimicrobial susceptibility test discs impregnated with known amount of antibiotics are placed on the agar surface. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

### Composition:

Ingredients	Grams/Litre
Beef heart infusion	2.0
Acid Casein Hydrolysate	17.5
Starch, soluble	1.5
Agar	17.0

Final pH 7.3 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

### Directions :

Suspend 38 g in 1000 ml of purified water. Heat with frequent agitation and boil for one minute. Sterilize at 121°C for 15 minutes. Cool to 50°C. Mix gently and dispense into sterile Petri dishes.

### Principle and Interpretation:

Mueller Hinton Agar 2 is recommended for the disk diffusion method of antibiotic susceptibility testing. Mueller Hinton medium is recommended by National Committee for Clinical Laboratory Standards (NCCLS) for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens. Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Chocolate Agar have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Thymine and thymidine inhibit Sulfonamide and Trimethoprim<sup>5,6</sup> activity and calcium and magnesium<sup>7,8</sup> interferes with activity of aminoglycoside antibiotics. To overcome this problems Mueller Hinton Agar 2 is manufactured to contain low levels of thymine, thymidine and controlled levels of calcium and magnesium.

Beef heart infusion and acid casein hydrolysate provide nitrogenous compounds, vitamins, carbon, sulphur, amino acids and other essential nutrients in Mueller Hinton media. Starch act as a "protective colloid" against toxic substances present in the medium.

The Bauer-Kirby procedure is based on the agar diffusion method. Paper discs impregnated with certain amount of a single antibiotic are placed on the surface of the medium. The plates are incubated and the zones of inhibition around each disc are measured. The inhibition zones correlate with the minimal inhibitory concentration (MIC). Different factors influence the disc diffusion susceptibility tests as inoculum concentration, agar depth, disc potency, medium pH and beta-lactamase production by test organisms.

Cultural characteristics after 24-48 hours at 35°C.

Organisms (ATCC)	Growth
<i>Escherichia coli</i> (25922)	+++
<i>Staphylococcus aureus</i> (25923)	+++
<i>Pseudomonas aeruginosa</i> (27853)	+++
<i>Neisseria meningitidis</i> (13090)	+++
<i>Streptococcus pneumoniae</i> (6305)	+++ (growth on Mueller Hinton Blood Agar)
<i>Streptococcus faecalis</i> (29212)	+++ (growth on Mueller Hinton Chocolate Agar)



## References:

1. J.H. Müller, J. Hinton, Proc. Soc. Exptl. Biol. 48, 330 (1941)
2. A. Bauer et al, Am. J. Clin. Path., 45, 493 (1966)
3. Present Status and Future Work, WHO Sponsored collaborative study, Chicago (Oct. 1967)
4. Ericsson and Sherris, Acta. Pathol. Microbiol., Scand. Sec. B. Suppl., 217, 1 (1971)
5. Koch and Burchall, Appl. Microbiol., 22, 812 (1971)
6. R. Ferone, et al, Antimicrob. Agents Chemother., 7, 91 (1975)
7. Pollock, Minshew, Kenny, et al, Antimicrob. Agents Chemother., 14, 360 (1978)
8. R.F. D'Amato, C. Thornsberry, Curr. Microbiol., 2, 135 (1979)
9. NCCLS Approved Standard: ASM-2, Performance Standards for Antimicrobial Disc Susceptibility Tests, 2<sup>nd</sup> ed., National Committee for Clin. Lab. Standards (1979)
10. NCCLS M7-T Tentative Standard, National Committee for Laboratory Standards (1983)
11. NCCLS, Performance Standards for Antimicrobial Disc Susceptibility Tests, 3<sup>rd</sup> ed., Approved Standard M<sub>2</sub>-A<sub>3</sub>, Villanova Pa (1984)
12. G.M. Eliopoulos, et al., Enhancement of cefotaxime and other cephalosporins against Enterococcus faecalis by blood supplemented Mueller-Hinton agar, Diagn. Microbiol. Infect. Dis. 12, 149 (1989);
13. R.D. Jenkins, et al., J. Clin. Microbiol. 22, 369 (1985)

## Precautions and Disclaimer

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